COMPARISON OF SEROLOGIC AND TAXONOMIC RELATIONSHIPS OF DROSOPHILA SPECIES*

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INTRODUCTION

During the past two years studies have been made of extracts prepared from various species of *Drosophila* with the view of distinguishing the species through the use of serological procedures. Several different technics have been tried, and the reagents which were tested have been prepared in different ways. The results of these experiments have been published in several reports, to which reference will be made later. The purpose of this work is to compare the species relationships, as revealed by the serologic investigations of their antigens, with the relationships which are recognized on the basis of the more commonly accepted taxonomic criteria.

Although the different serological tests have yielded similar results, seldom have they offered exactly the same relations among the several species. It is impossible, at the present time, to state with certainty which technic reveals most nearly the real biochemical relationships among the species. Boyden (1936) has stated that no two technics are of equal worth; and Chester (1937), in his extensive review of plant serology, has been unable to conclude which of the many technics and modifications most nearly reveal the truth. This lack of agreement of the various tests and the consequent indecision regarding the relative value of serologic technics present a problem not greatly different from that of the taxonomist. The taxonomist is confronted with the difficulty of determining which characteristic or group of characteristics relates most truly the various species.

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Aside from the difficulty in appraising the systematic worth of any morphological characteristic, another problem of species inter-relations presents itself. A given characteristic which is thought to be of specific value may serve well to distinguish one species from a second, but may in turn be shared by a third species. This fact, no doubt, has contributed considerably to the confusion regarding our knowledge of evolution and inter-relations of species. In serological investigations of animals relationships the same feature has been noted by Irwin (1938), who found that each dove or pigeon species possessed cellular substances, determined by the agglutinin-absorption technic, which were not found in any of the other species. Also, he showed that some of the serological characteristics which distinguished one species from another could in turn be shared with still other species. Hence, difficulties of evaluating specific qualities are common to both the morphologic and immunologic procedures. In serologic tests, however, the reactive substances are biochemical elements of the body tissues of the animal. Through the application of technics which assay these fundamental chemical properties of the organism, we should be able ultimately to determine with a reasonable degree of exactitude the extent to which the protoplasm of one species is similar to that of others.

MATERIALS AND METHODS

In the serological tests that have been performed the relationships of about a dozen *Drosophila* species have been studied. Extensive data, however, have been accumulated only for the four species: *D. caribbea, D. melanogaster, D. mulleri,* and *D. virilis.* At least a dozen tests, representing several different procedures, have been performed on the antisera to each of these species. The taxonomic data used in this report were taken largely from Professor Sturtevant's work (1921). These data were supplemented by those of other authors (Metz, Moses, and Mason, 1923) and by some of my own observations. Recognizing our incapacity to appraise the relative specific worth of any morphological unit or serological reaction, I have assumed that all of the various immunological tests are of equal value, and that all of the fourteen taxonomic characters are of equal value.

The serological reactions which were employed in this comparison were as follows:

1. Complement-fixation reactions, using as antigens the saline extracts of dried flies, without any further extraction (Cumley and Haberman);
2. Precipitation reactions—ring tests, using the same antigens as in the preceding (Haberman and Cumley);
3. Complement-fixation reactions, using as antigens the saline extracts of the ether-insoluble fractions of dried flies (Cumley, 1939);
4. Optimal antigen-antibody ratio reactions, using the same antigens as in the tests immediately above (Cumley, 1938);
5. Precipitin absorptions, using the same antigens as in the preceding (Cumley, 1939, a).

The systematic characteristics employed in this comparison were as follows:

1. Costal index: Length of 2nd section of costal vein/Length of 3rd section of costal vein;
2. Fourth-vein index: Length of 4th (distal) section of 4th vein/Length of 3rd section of 4th vein;
3. 4c index: Length of 3rd section of costal vein/Length of 3rd section of 4th vein;
4. 5x index: Length of 3rd (distal) section of 5th vein/Length of posterior vein;
5. Number of branches of arista;
6. Approximate width of the "front"/width of the head;
7. Size of 2nd orbital bristle/Size of the other two;
8. Size of the 1st oral bristle/Size of the 2nd;
9. Greatest width of the cheeks/Greatest diameter of the eyes;
10. Number of rows of acrostichal hairs;
11. Number of filaments on eggs;
12. Body length;
13. Wing length;
14. Number and type of chromosomes.

In order to rank the species serologically, each serologic test was considered separately, and the species were arranged in the order of the extent of reactivity of their antigens to the particular antiserum in question. This procedure was repeated for each

of the several tests. Since all the tests of a given antiserum did not rank the species in the same order, the result was that any one species would sometimes assume second, and sometimes third or fourth place. From data taken in this way from the various tests, it was possible to observe the number of times a particular species assumed each of the four serological ranks made possible by a consideration of only four species. The percentage ranks were calculated from these observations and are shown in Table 1.

TABLE 1

SEROLOGIC RANKING

Species for comparison	Percentage ranks			
	1	2	3	4
Species in question: *Drosophila melanogaster*				
D. melanogaster	100.0	0	0	0
D. caribbea	0	96.5	3.5	0
D. mulleri	0	3.5	64.3	32.2
D. virilis	0	0	32.2	67.8
Species in question: *Drosophila caribbea*				
D. caribbea	100.0	0	0	0
D. melanogaster	0	71.1	13.5	15.4
D. mulleri	0	11.5	57.8	30.7
D. virilis	0	17.4	28.7	53.9
Species in question: *Drosophila mulleri*				
D. mulleri	100.0	0	0	0
D. virilis	0	88.5	11.5	0
D. caribbea	0	7.7	69.3	23.0
D. melanogaster	0	3.8	19.2	77.0
Species in question: *Drosophila virilis*				
D. virilis	100.0	0	0	0
D. mulleri	0	82.2	3.6	14.2
D. caribbea	0	17.8	50.0	32.2
D. melanogaster	0	0	46.4	53.6

In this table one may observe that, with reference to *D. melanogaster,* the four *Drosophila* species are ranked as follows: *melanogaster,* 1st place in 100 per cent of the tests; *caribbea,* 2nd place in 96.5 per cent, and 3rd place in 3.5 per cent of the tests; *mulleri,*

2nd place in 3.5 per cent, 3rd place in 64.3 per cent, and 4th place in 32.2 per cent of the tests; *virilis,* 3rd place in 32.2 per cent and 4th place in 67.8 per cent of the tests. The same type of information is to be seen in the remainder of the table. That is to say, all four of the species are ranked in their respective relations to a given species.

The taxonomic ranking has been accomplished in much the same way, except that instead of considering serological tests, the individual morphological units have been applied. The species are then ranked in the order of their relationship in terms of a given systematic criterion. As in the preceding rankings, the percentage ranks of the various species with reference to a given species have then been calculated. These percentage ranks have been presented in Table 2. In this table one may see that the

TABLE 2

TAXONOMIC RANKING

Species for comparison	Percentage ranks			
	1	2	3	4
Species in question: *Drosophila melanogaster*				
D. melanogaster	100.0	0	0	0
D. caribbea	0	77.0	23.0	0
D. mulleri	0	7.6	42.4	50.0
D. virilis	0	15.4	34.6	50.0
Species in question: *Drosophila caribbea*				
D. caribbea	100.0	0	0	0
D. melanogaster	0	84.8	11.5	3.7
D. mulleri	0	7.6	46.2	46.2
D. virilis	0	7.6	42.3	50.1
Species in question: *Drosophila mulleri*				
D. mulleri	100.0	0	0	0
D. virilis	0	69.3	7.6	23.1
D. melanogaster	0	11.5	53.8	37.7
D. caribbea	0	19.2	38.6	42.2
Species in question: *Drosophila virilis*				
D. virilis	100.0	0	0	0
D. mulleri	0	77.3	4.5	18.2
D. melanogaster	0	9.1	59.1	31.8
D. caribbea	0	13.6	36.4	50.0

various *Drosophila* species are related to *D. melanogaster* as follows: *melanogaster,* 1st place in 100 per cent of the characters; *caribbae,* 2nd place in 77.0 per cent and 3rd place in 23.0 per cent of the characters; *mulleri,* 2nd place in 7.6 per cent, 3rd place in 42.4 per cent, and 4th place in 50.0 per cent of the characters; and *virilis,* 2nd place in 15.4 per cent, 3rd place in 34.6 per cent, and 4th place in 50.0 per cent of the taxonomic characters. Similarly, all four of the species are ranked taxonomically in their relations to a given species.

RESULTS

Figure 1 has been prepared in order that a more graphic view may be obtained of the comparison of the serologic and taxonomic rankings. From a perusal of this figure and the data of Tables 1 and 2 several facts become apparent:

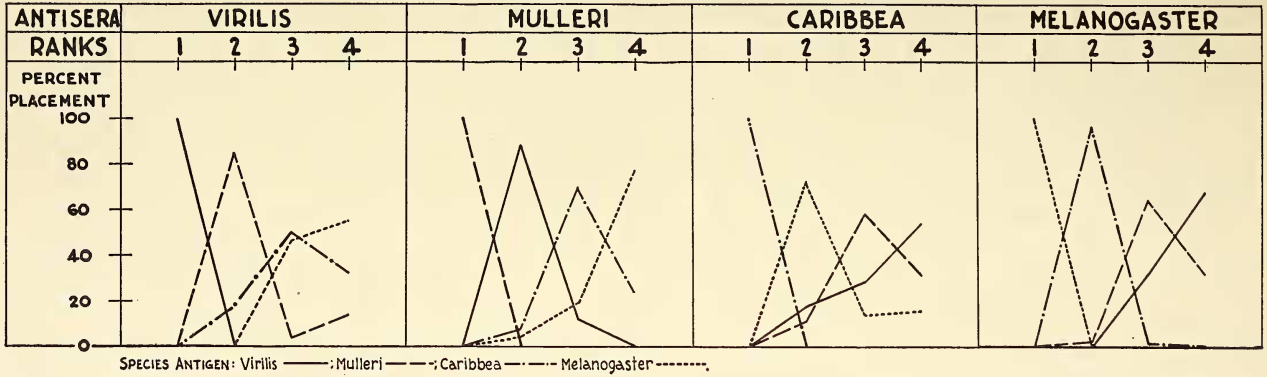
1. The serological and taxonomic technics have always presented the same species for second rank.

2. With reference to ranks three and four, the taxonomic and serologic methods have given essentially the same results in two of the comparisons (*D. melanogaster* and *D. caribbea*), and have failed to correspond, apparently, in the other two comparisons. In the two cases where the results failed to coincide, however, there is some doubt as to the real ranking on the basis of taxonomic data. That is to say, when the species were ranked taxonomically in relation to *D. virilis,* the species *caribbea* assumes fourth rank in 50.0 per cent of the characteristics, whereas *melanogaster* ranks fourth in only 31.8 per cent of the tests. By virtue of these figures alone, *caribbea* obviously would receive fourth rank. However, *caribbea* also ranks second in 13.6 per cent of the systematic features, whereas *melanogaster* ranks second in only 9.1 per cent. Therefore, it is impossible to determine offhand which of the two species should be considered as ranking third place. The same situation exists when the species are given morphological ranks, with reference to *D. mulleri.*

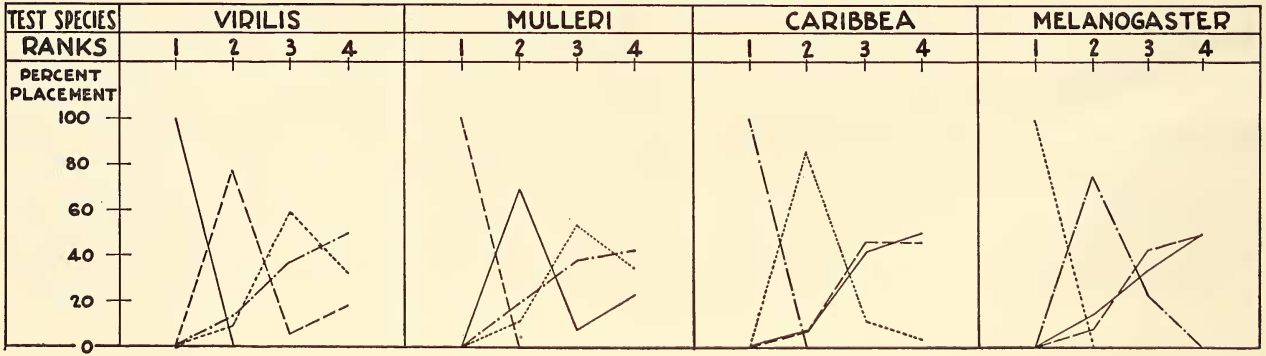
In general, then, we may conclude that the taxonomic and serologic methods agree definitely to the extent of the second rank, and when they disagree as to the third and fourth ranks, the taxonomic method has not clearly indicated which order of relationship is correct.

FIGURE 1

SEROLOGIC RANKING OF DROSOPHILA SPECIES



TAXONOMIC RANKING OF DROSOPHILA SPECIES



3. The results of the serologic ranking apparently are more specific than are those of the taxonomic ranking. This fact is indicated by several features of Figure 1 and Tables 1 and 2. First, a species which ranks third serologically always is represented by a sharply-peaked curve. Not only is that not the case with the taxonomic ranking, but there is always some doubt as to which species really ranks third. This fact is readily apparent since all of the curves representing the third and fourth places, taxonomically, cross each other or coincide in at least three points; whereas in the curves representing the serologic ranks, only twice do the 3rd and 4th rank curves coincide more than once. Furthermore, in none of the taxonomic rankings, as observed in Table 2, is it possible to determine which species ranks third and which fourth; whereas in the serologic data of Table 1, in only one ranking (*caribbea*) is this the case. Second, in two of the serological rankings those species which rank fourth never ranked second in any of the tests. In the case of the taxonomic ranking, all the species that ranked fourth on the basis of some morphological unit, ranked second on the basis of some other. Third, those species which are ranked fourth serologically are so ranked by virtue of from 53.6 per cent to 77.0 per cent of the tests indicating this rank. Those species ranked fourth taxonomically are so ranked by virtue of 42.2 per cent to 50.1 per cent of their characteristics. Similar figures may be observed for the second and third ranks.

4. The two subgroups designated by Sturtevant (1921) as subgroup 1 and subgroup 2 of Group F have been definitely indicated by the serologic methods, as well as by the taxonomic studies. Sturtevant's subgroup 1 includes the yellowish or reddish species of which *D. melanogaster* and *D. caribbea* are members. Subgroup 2 includes the blackish or grayish species of which *D. virilis* and *D. mulleri* are members. In the serologic ranking as well as in the morphologic ranking, *D. virilis* and *D. mulleri* always rank close together, and *D. caribbea* and *D. melanogaster* always rank close together. If one assumes that these two subgroups arose from a common stock and that the species of each subgroup diverged much later, then this is more or less the relation one would expect to find.

CONCLUSIONS

Serologic technics recently have been employed by several investigators in relating or ranking species of molluscs (Makino, 1934), helminths (Eisenbrandt, 1936), amphibia (Boyden and Noble, 1933), moths (Martin and Cotner, 1934), and other animal species. Problems of hybrids, likewise, have been attacked by these biochemical methods (Irwin, 1938; Irwin and Cole, 1936 a & b; Irwin, Cole and Gordon, 1936). In general the results have been sufficiently encouraging to warrant the application of serologic technics to an analysis of *Drosophila* species. The present paper and those which have preceded serve to indicate the results which may be expected from such procedure. Obviously, the work has only begun, and several refinements are necessary. It is believed, however, that these methods eventually will provide valuable data relative to problems of speciation and phylogeny. Furthermore, the recent work of Levit, Ginsburg, Kalinin, & Feinberg (1936) suggests the possibility of applying immunologic technics to the study of the expression of individual chromosomes or even genes. To what extent the method may be utilized remains at present a matter of conjecture.

These comparisons have demonstrated that on the basis of morphology, species cannot always be ranked to the third and fourth places, but with the serologic methods this can be accomplished. In cases where morphological differences are insignificant or absent, the serologic technics may provide adequate means of determining species relationships.

LITERATURE CITED

Boyden, A. A. 1936. Serology and biological problems. Sigma Xi Quar., 24: 152–160.

Boyden, A. A., and Noble, G. K. 1933. The relationships of some common Amphibia as determined by serological study. American Museum Novitates. No. 606, pp. 1–24.

Chester, K. S. 1937. A critique of plant serology: Part I, The nature and utilization of phytoserological procedures, Quar. Rev. of Biol., 12: 19–46; Part II, Application of serology to the classification of plants and the identification of plant products. *Ibid.*, 165–190; Part III, Phytoserology in medicine and general biology,—bibliography. *Ibid.*, 294–321.

Cumley, R. W. 1937. Serology of *Drosophila*. Rec. of the Genetics Soc. of America., No. 6, p. 146 (Abstract).

———. 1938. Serological Investigation of *Drosophila* Antigens. Dissertation, The University of Texas.

———. 1939. The relations among *Drosophila* species, as determined by the complement fixation reaction using ether-insoluble fractions. Jour. Exp. Zool., 80: 299–314.

———. 1939, a. Precipitin absorption with *Drosophila* antigens. The American Naturalist, Vol. LXXIII, pp. 375–380.

CUMLEY, R. W., AND HABERMAN, S. 1938. Serological investigation of *Drosophila* antigens with the complement fixation reaction. Jour. N. Y. Ent. Soc., Vol. XLVI, pp. 401–415.

EISENBRANDT, L. L. 1936. Precipitin reactions of Helminth extracts. Proc. Soc. Exp. Biol. and Med., 35: 322–325.

HABERMAN, S., AND CUMLEY, R. W. 1939. Serological investigation of *Drosophila* antigens using the precipitation reaction. Jour. N. Y. Ent. Soc., Vol. XLVII, pp. 219–226.

IRWIN, M. R. 1938. Immuno-genetic studies of species relationships in Columbidæ. Jour. of Gen., 35: 351–373.

IRWIN, M. R., AND COLE, L. J. 1936a. Immunogenetic studies of species and of species hybrids in doves, and the separation of species-specific substances in the backcross. Jour. Exp. Zool., 73: 85–108.

———. 1936b. Immunogenetic studies of species and of species hybrids from the cross of *Columba livia* and *Streptopelia risoria*. Jour. Exp. Zool., 73: 309–318.

IRWIN, M. R., COLE, L. J., AND GORDON, D. D. 1936. Immunogenetic studies of species and of species hybrids in pigeons, and the separation of species-specific characters in back-cross generations. Jour. Exp. Zool., 73: 285–308.

LEVIT, S. G., GINSBURG, S. G., KALININ, V. S., AND FEINBERG, R. G. 1936. Immunological detection of the Y-chromosome in *Drosophila melanogaster*. Nature, 138; 3480; 78–79.

MAKINO, K. 1934. Beobachtungen uber die Immunitatsreaktionen bei Molluskenarten. Zeit. fur Immunitats. Bd. 81, Heft 3/4, 316–335.

MARTIN, S., AND COTNER, F. B. 1934. Serological studies of moth proteins with special reference to their phylogenetic significance. Ann. Ent. Soc. Amer., 27: 372–383.

METZ, E. W., MOSES, M. S., AND MASON, E. D. 1923. Genetic studies on *Drosophila virilis* with considerations on the genetics of other species of *Drosophila*. Carnegie Institution of Washington. Publ. No. 328, 94 pp.

STURTEVANT, A. H. 1921. The North American Species of *Drosophila*. Carnegie Institution of Washington. Publ. No. 301, iv + 150 pp.